GGCTCAGCATGGCGCGCTTCAGGCCAATCAGGCTCAGCACGCGGGGCATGCCGC GCTTCAGCATGTTCACGGCGCGGCTCTTGCCGGG (SEQ ID NO:16).

Primer 4. sIgh-VChU2+ (90mer)

GGCCTGAAGCGCGCCATGCTGAGCCTGATCGACGGCAAGGGCCCCATACGCTTC GTGCTGGCCCTGCTGGCCTTCTTCCGCTTCACCGCC (SEQ ID NO:17).

Primer 5. sIgh-VChL2- (89mer)

GGTGCTTCATGGCGGTCTGCTTGTTCACGCCGCGCCAGCGGTCCAGCACGGCGCG GGTGGGGGCAATGGCGGTGAAGCGGAAGAAGGCC (SEQ ID NO:18).

Primer 6. sIgh-VChU3+ (89mer)

CCGCCATGAAGCACCTGCTGAGCTTCAAGAAGGAGCTGGGCACCCTGACCAGCG CCATCAACCGCCGCAGCAGCAAGCAGAAGAAGCGC (SEQ ID NO:19).

Primer 7. sIgh-VChL3- (81mer)

CGCGCCCACGCTGGCGATCAGGCCAATCATCACGGCAATGCCGGTCTTGCCGCC GCGCTTCTTCTGCTTGCTGCTGCGGCG (SEQ ID NO:20).

Primer 8. sIgh-VChFS1+ (39mer)

CCCAAGCTTGCCGCCACCATGGACTGGACCTGGATCCTG (SEQ ID NO:21).

Primer 9. sIgy-VCyFS1.1+ (33mer)

CCCAAGCTTGCCGCCACCATGGATTGGACTTGG (SEQ ID NO:22).

Primer 10. sIgh-VChFAS2- (37mer)

ATAGTTTAGCGGCCGCGCCCACGCTGGCGATCAGGCC (SEQ ID NO:23).

Three sets of primers were paired for PCR reactions to generate three overlapping PCR products as follows: primers 1 and 3 (for pWNVh-DJY) or primers 2 and 3 (for pWNVy-DJY), primers 4 and 5, and primers 6 and 7. Each set of primers was self-annealed and extended by *Pfu* DNA polymerase (Stratagene, La Jolla, CA). The final, full-length inserts were amplified with a primer set of primers 8 and 10 (to generate the insert for pWNVh-DJY) or primers 9 and 10 (to generate the insert for pWNVy-DJY), and subsequently tailed with HindIII (5' end) and NotI (3' end) endonuclease restriction sites. These final insert products were restricted with HindIII and NotI, and cloned into HindIII/NotI-digested pcDNA3.1/V5-HisC. The resultant recombinant vectors (pWNVh-DJY and pWNVy-DJY) were confirmed by sequencing. Figures 2 and 5 present the restriction endonuclease maps of pWNVh-DJY and pWNVy-DJY, respectively. Figures 3 and 6 present the feature maps of pWNVh-DJY and pWNVy-DJY,

respectively. Figures 4 and 7 present the complete, annotated nucleotide sequences for pWNVh-DJY and pWNVy-DJY, respectively.

Example 2: Biological Characterization of WNV Capsid Protein Expressed from pWNVy-DJYand pWNVh-DJY.

Expression of Cp protein from pWNVy-DJY and pWNVh-DJY in tissue culture cells

HeLa, RD, or 293 cells, seeded onto two-chamber slides, were transfected by the $CaPO_4$ precipitation method with 2μg of purified plasmid DNA (either pWNVy-DJY or pWNVh-DJY). Following transfection, the cells were fixed cells and incubated with mouse anti-His mAb and then incubated with FITC-conjugated goat anti-mouse IgG Ab. The gene expression was examined with UV lamp microscope. Expression of Cp protein was achieved in all three cell lines from both constructs pWNVy-DJY and pWNVh-DJY, and the protein was localized in the cytoplasm. Immunofluoresence analysis of the expression of Cp protein in RD cells transfected with pWNVh-DJY revealed a green signal representing localized Cp protein using a FITC filter. The images were also captured with a dual filter of FITC and rhodamine to distinguish between specific and background signals. Green fluorescence under the dual filter confirmed the presence of Cp protein. A DAPI filter was used to reveal the nuclei of the cells, which were stained with DAPI (4', 6-diamidine-2'-phenylindole, dihydrochloride), and cellular morphology was revealed when the image was captured with a DAPI filter in the light field.

***In vitro* translation of WNV capsid protein**

^{35}S-labeled protein products were prepared using the TNT-T7 coupled Transcription/Translation System (Promega, Madison, WI). Ten μl of radiolabeled protein samples and 1 μl of anti-His (C-term) (Invitrogen, San Diego, CA) antibody were added to 300 μl of RIPA buffer and mixed gently. After an incubation at 4°C for 90 minutes, Protein A-Sepharose beads (LKB-Pharmacia Biotech) were added to the protein-antibody complexes at a final concentration of 5 μg per tube and the samples were incubated at 4°C for 90 minutes in a rotating shaker. The beads were washed three times with RIPA buffer and suspended in 2X SDS sample buffer. The immunoprecipitated protein complexes were eluted from the Sepharose beads by brief boiling and resolved in SDS-PAGE (15%) gels. The mobilities of the protein samples were compared with that of commercially available, ^{14}C-methylated molecular weight markers (Sigma). The gel was fixed, treated briefly with 1M sodium salicylate solution and

dried in a gel dryer (BioRad). The dried gel was exposed overnight to X-ray film (Kodak). The *in vitro* translated proteins had an apparent molecular size of 21.5 kDa (Figure 8).

Example 3: Evaluation of Immune Response Against WNV Capsid Protein Expressed from pWNVy-DJY and pWNVh-DJY.

Peptides

Three major histocompatibility (MHC) class II-restricted epitopes of the WNV Cp amino acid sequence were chosen using MacVector software (Oxford Molecular Group, MA), which is capable of predicting antigenic determinants and hydrophilic regions. The peptides were synthesized by standard peptide synthesis, and were as follows:

Peptide Name	WNV Cp Protein Residues	Amino Acid Sequence	SEQ ID NO
WNVC-P1	2 - 23	SKKPGGPGKSRAVNMLKRGMPR	SEQ ID NO:6
WNVC-P2	31 - 49	KRAMLSLIDGKGPIRFVLA	SEQ ID NO:7
WNVC-P3	90 - 111	TLTSAINRRSSKQKKRGGKTGI	SEQ ID NO:8

Figure 8 presents these peptides aligned along the length of the WNV Cp protein.

***In vitro* translated protein**

Non-radioactive, *in vitro* translated Cp protein was also generated as described above in Example 2, using the TNT-T7 coupled Transcription/Translation System (Promega, Madison, WI) with non-radioactive components. An *in vitro* translation control was generated using the *in vitro* translation kit with the pcDNA3.1 vector (Invitrogen, San Diego, CA), lacking an expressible insert.

DNA inoculation of mice

To evaluate the T cell-mediated immune response against the WNV Cp gene product, an *in vivo* mouse experiment was set up. The quadriceps muscles of 6- to 8-week-old female BALB/c mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were injected with 100 μg of pWNVh-DJY, pWNVy-DJY, or pcDNA3.1 (without an insert) in PBS and 0.25% bupivacaine-HCl (Sigma, St. Louis, MO). Two weeks later, the mice received a boost of another 100μg DNA injections. Thirteen days after the boost injection, the mice were sacrificed, the spleens were harvested, and the lymphocytes were isolated and tested for cellular immune responses.